

Improvement Method in Maize (*Zea mays* L) using Technology of Haploid Induction *in vivo* with Conventional Methods

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Abstract: *The objective of any improvement program is the increase in yields, this leads the breeders to continually rethink the methods to increase their efficiency and thus obtain results in less time. This paper describes the possibility of combining, for the improvement of maize, a new breeding technology, such as induction of haploidy in vivo, with two traditional breeding methods such as: the cryptic hybrid method and the zygote selection method, for the fast and efficient provision of double haploid (DH) lines, with the advantage of knowing their specific combining ability, providing valuable information of the DH lines, before their final development.*

Keywords: Maize, double haploids, cryptic hybrids, zygote selection

1. Introduction

The aim of plant improvement is the increase in crop yields, which is why the breeders continually rethink the methods used, especially their efficiency when evaluating the high costs and the results obtained. Duvick, et al. (2004) indicates that with the current techniques the cost per unit of yield gain has increased continuously in recent years and will probably continue to increase, unless other more efficient methods are introduced. As consequence of this, new procedures for the improvement of populations originated, which seek to improve efficiency, reducing the time to obtain inbred and hybrid lines (Toppa *et al.*, 2012).

The aim of this review is to describe the possibility of combining, in maize, haploid induction technology *in vivo*, with two traditional improvement methods, such as cryptic hybrids method (Hallauer, 1967; Lonquist and Williams, 1967), and the zygote selection (Hallauer, 1970).

The "cryptic" hybrids method

The "cryptic" hybrids method was proposed by Hallauer (1967) and Lonquist and Williams (1967) to obtain superior hybrids. It consists of selecting individual prolific plants within the heterotic groups, self-fertilizing (S1) and reciprocally crossing (F1). Evaluate crossings in field yield trials, based on which the self-fertilized lines are selected.

Villena (1965) explains that this procedure allows to identify very early the lines whose genotypes in specific crosses express a high degree of heterosis and to decide the use of these lines in a genetic improvement program.

The important thing to do the self-fertilization in the two plants of each cross plant to plant, is that the crosses or hybrids thus obtained can be reproduced later, since "theoretically the gene frequencies of the populations derived from the self-fertilized cobs must be similar". Consequently, the behavior of the crosses between

populations derived from the self-fertilized cobs should be similar to the behavior of the original plant-to-plant crosses.

The method is based on increasing the frequency of complementary genes and obtaining lines with good SCE (Botega Alves *et al.*, 2012; Gomes *et al.*, 2005). This maximizes the selection by SCA (Farias, *et al.*, 2008), which is indicated by Santos *et al.*, (2007) as the most exploited effect on maize improvement.

Cryptic hybrids have limited use in breeding programs, mainly due to the lack of information to assess their potential. This prevents it from becoming commonly used to obtain hybrids (Gomes Lopes *et al.*, 2001).

Zygote selection

The zygote selection is a method of improvement proposed by Hallauer (1970), as a modification of the gamete selection (Stadler, 1944), it is based on the identification of higher genotypes of an original population, it consists of self-fertilization (S1) and crossing with a line elite (F1), used as tester, of the selected plant.

Double haploid technology *in vivo* in maize

Double haploid (DH) technology in maize improvement, based on the induction of haploidy *in vivo*, is an important technology to increase the efficiency of breeding (Prasanna *et al.*, 2013)

This method consists of crossing selected plants from a source population with an inductor line. This haploid induction technology *in vivo* was adopted by several commercial maize improvement programs in Europe (Schmidt, 2003), North America (Seitz, 2005) and China (Chen *et al.*, 2009).

The main advantages offered by this technology are: first that completely homozygous lines are obtained and second the selection cycle is considerably shortened, achieving in only two generations, which would traditionally take at least

eight generations, which is equivalent to at least four years, doing two cycles per year.

Obtaining DH lines *in vivo* is relatively simple. Thanks to the efforts of researchers such as Coe (1959) and Coe and Sarkar (1964), who identified haploid-inducing maize lines, and incorporated an anthocyanin-based phenotypic color marker called the R1-Navajo (R1-nj) gene. This is expressed in the aleurone (external part of the maize endosperm) and in the embryo (scutellum) of the haploidy inducer, unlike the source populations, which usually have no anthocyanin coloration in the embryo or endosperm. Therefore, the gene, R1-nj as a dominant color marker, helps differentiate haploid grains (n), without purple or red color in the scutellum, but with coloration in the endosperm crown, while diploid grains (2n) have color purple or red, both in the endosperm and in the skullcap (Nanda and Chase, 1966; Greenblatt and Bock, 1967; Chase, 1969).

Description of the proposed improvement methodology

The first method proposed is to use the technology of DH, with the method of cryptic hybrids. In which in populations of different heterotic groups for the generation of hybrids, which is from prolific plants, such as those used in the method of cryptic hybrids proposed by Hallauer 1967, and Lonnquist and Williams (1967), but instead of self-fertilize and cross-select between selected plants of the different heterotic groups, the latter is done, to obtain the F1 and the other ear, instead of self-fertilizing, it would be pollinated with a haploidy-inducing line, as outlined in the Figure 1. Crossings should be done manually and identified. With the F1 the field yield trials are made, while the DH-induced seeds would be labeled and stored, for their subsequent treatment to obtain the DH lines, which would be done based on the data of the F1 tests. This would be an early evaluation by specific combining ability (SCA), which is important in the development of hybrids.

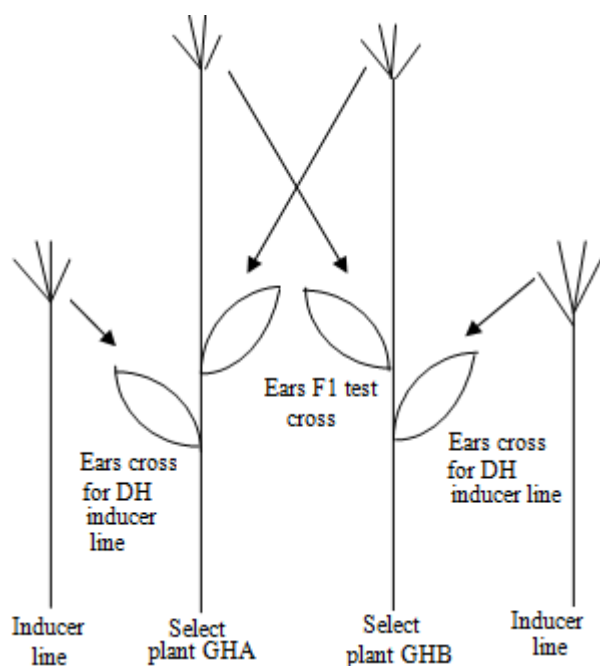


Figure 1: Scheme of the first proposed method, with "cryptic" hybrids method

The second method proposed is the use of DH technology in a source population, following the steps of the zygote selection proposed by Hallauer (1970), crossing the inducer with the selected plant, whose seeds would be identified and stored, and at the same time with the pollen of these selected plants, pollinate tester lines for the production of F1, are identified and used as testers in field trials (Figure 2). In this case the crossings should also be done manually, as in the previous proposal.

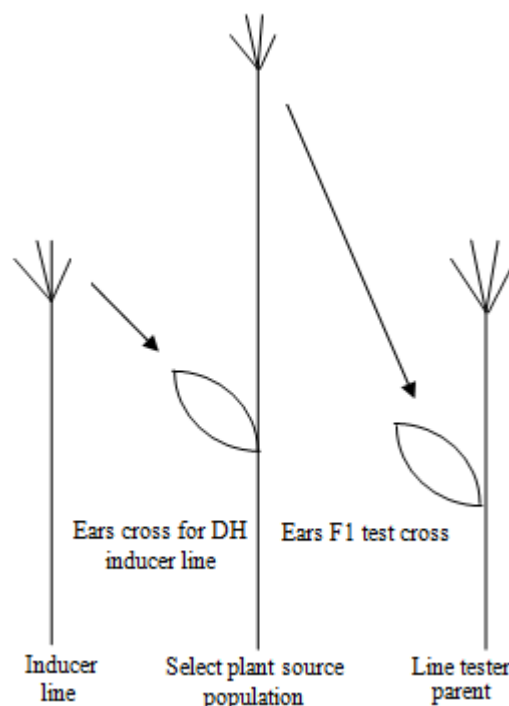


Figure 2: Scheme of the second proposed method, with zygote selection.

These proposals have the advantage that was expressed by numerous authors such as Jenkins (1935), who points out that the combining ability of the lines is fixed at stages as early as S1. Suwantaradom and Eberhart (1974) who conclude that early testing of S1 line hybrids is effective. Rodríguez and Hallauer (1991) point out that identifying families of complete siblings of low yield is effective, since it allows them to be discarded early. On the other hand, Horner (1963) points out that better discrimination between genotypes should be possible when the S1 plant, instead of the S1 line, is evaluated by combining ability, whether or not it is chosen at random. Hallauer (2007) based on the results obtained by several researchers, says that apparently the concept of the early Jenkins and Sprague tests has been validated.

2. Conclusion

These methods described above. The first could be used in populations where the DH method is already being applied, from different heterotic groups *per se*, and to obtain lines that give good hybrid combinations, also the populations that form the heterotic groups could be maintained in isolated lots with free polization, without losing its genetic variability. The second could be used with germplasm exotic or germplasm bank accessions, to identify higher genotypes, or select lines for hybrid combinations

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